



CASE: LA0112 NP

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Burton Rodney
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Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

ART UNIT: 1626

TIMUR GUNGOR, ET AL.

EXAMINER: STOCKTON, LAURA LYNNE

APPLICATION NO: 10/775,742

FILED: 02/10/2004

FOR: NOVEL THIAZOLIDINE COMPOUNDS AS CALCIUM
SENSING RECEPTOR MODULATORS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION OF RAMAKRISHNA SEETHALA
TO OVERCOME CITED U.S. PATENT NO. 6,673,821

To the Commissioner for Patents and Trademarks:

1. This Declaration is to establish reduction to practice of the invention in this application at a date prior to October 22, 2001, that is the filing date of U.S. application Serial No. 10/007,342, now U.S. Patent No. 6,673,821.
2. Ramakrishna Seethala declares as follows.
3. He was awarded a Ph.D. in Biochemistry.

4. He has been employed by Bristol-Myers Squibb Company, the assignee of the subject application as a biologist, for 16 years, about four years of which as a supervisor in the Department of Metabolic Diseases, Age Related Diseases and Bone Biology.

5. That prior to October 22, 2001, a sample of the compound of Example 1 (identified as BMS 515,832) of the subject application was received by Dr. Ramakrishna Seethala for testing of such compound as a modulator of the calcium sensing receptor.

6. Prior to October 22, 2001, he was requested to test the compound of Example 1 (BMS 515,832) of the subject application for its activity as a modulator of the calcium sensing receptor.

7. Prior to October 22, 2001, he requested Zhengping Ma, a biologist working under his supervision, to carry out experiments to test the compound of Example 1 (BMS 515,832) for its activity as a modulator of the calcium sensing receptor.

8. The calcium receptor inhibitor assay employed for such testing was Protocol CaR_H_IC50 which is as follows:

Calcium Receptor Inhibitor Assay Methods:

Inhibition of intracellular calcium:

Calcilytic activity was measured in human TT cells (ATCC No. CRL-1083) by determining the IC₅₀ of the test compound for blocking increases in intracellular Ca²⁺ by extracellular Ca²⁺ (as agonist of the receptor). Intracellular Ca²⁺ was measured using Fluo3,AM (Molecular probes, # F-1242) as indicator dye. Intracellular Ca²⁺ increase was measured with extracellular Ca²⁺ from 0.5 to 5 mM in Fluorescence Imaging Plate Reader (FLIPR) (Molecular Devices).

The Ca²⁺ receptor inhibitor assay procedure is as follows: TT cells were maintained in T-150 flasks in cell growth medium (F-12K Nutrition Media (Gibco 211270-022) with 10% heat

inactivated FBS, and 1x Glutamax) in 5% CO₂:95% air at 37°C to 90% confluency. The medium was removed, the cell monolayer was washed with phosphate buffered saline (PBS), incubated with 0.05% trypsin at 37°C for 2 minutes and the cells were dispensed by agitation. Cells from 2 flasks were pooled and centrifuged (200xg). The cell pellet was suspended in cell growth medium. Cells were plated 30,000 cells/well for 2 days, or 24,000 cells/well for 3 days in 96-well black view plates (Falcon, VWR#624-06-468) and incubated in 5% CO₂:95% air at 37°C. Cell medium was aspirated, and cells were loaded with Fluo3 (Molecular Probes, 50 µg dissolved in 25 µl DMSO, 50 µl 20% Pluronic Acid) in base buffer (10 mM HEPES buffer containing 1x Hank's salt, 0.1% BSA, 0.05% D-glucose, 0.8 mM CaCl₂) or 1 hour in a 37°C incubator. After incubation, loading buffer was aspirated and 120 µl/well base buffer was added.

Drug plates were prepared in base buffer and loaded into FLIPR. 30 µl from drug plate was added to the cell assay plate and fluorescence signals were read in FLIPR. Drug plate was replaced with CaCl₂ plate in FLIPR plate draw and 30 µl CaCl₂ (1.7 mM final for IC₅₀s, or 2.0 mM for screening) was added into cell plate by FLIPR. The fluorescence signal was measured by reading at 1 second intervals for 30 seconds and at 3 second intervals for the next 150 seconds. Calcilytic activity of the compounds was measured by their ability to block, in a concentration dependent manner (half-log concentrations in triplicate), the intracellular Ca²⁺ level by extracellular 1.7 mM Ca²⁺. The data was processed by ActivityBase (IDDBS) and the IC₅₀ values are determined by protocols developed.

9. Prior to October 22, 2001, Zhengping Ma, working under the supervision of Dr. Ramakrishna Seethala, conducted experiments wherein the Example 1 compound identified as BMS 515,832 was tested for its activity as a modulator of the calcium sensing receptor (Ca R response in TT human cells) and recorded the test results in Notebook No. 49513 pages 079-081, 083 and 084. (ATTACHMENTS K through Q)

10. A summary of the test results obtained by Zhengping Ma prior to October 22, 2001, and recorded in Notebook No. 49513, pages 079-081, 083 and 084, working under the supervision of Dr. Ramakrishna Seethala, is set out in a summary sheet (ATTACHMENT R) prepared after October 22, 2001. As seen in the summary sheet, the Example 1 compound was tested as per the

above protocol for its activity as a modulator of the calcium sensing receptor and was found to have such activity.

11. The actual dates of Experiments regarding the preparation of the Example 1 compound recorded in Notebook No. 49,513, pages 079-081, 083 and 084 were carried out and the dates of signing by Zhengping Ma and witnessing by Yong Quan, were all prior to October 22, 2001, but have been obliterated.

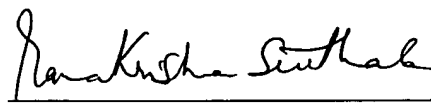
12. Portions of Notebook No. 49,513, pages 079-081, 083 and 084 have been obliterated because they were not relevant to the conception and reduction to practice of the present invention as it concerns Example 1 compound.

13. The above clearly establishes conception and reduction to practice of the invention covered by the relevant claims of the subject patent application (vis-à-vis U.S. Patent No. 6,673,821) prior to October 22, 2001.

14. This Declaration is submitted prior to Final Rejection.

15. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application Serial No. 10/775,742 or any patent issued thereon.

Date: 9/20/2006


RAMAKRISHNA SEETHALA

BRISTOL-MYERS SQUIBB

NOTEBOOK No. 49513

Assigned to

Zhenyong Ma

Subject

Department Name

Aging Research

Department Number

800 1602

Date Assigned

Date Completed

Pages Completed from

021

to

250

Continued from Notebook Number

49423

Continued in Notebook Number

51731

Caution

Proprietary and confidential information of Bristol-Myers Squibb Company.
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ATTACHMENT K

49513

TABLE OF CONTENTS

| PROJECT OR EXPERIMENT NO. | PRODUCT OR SUBSTANCE | STUDY PERFORMED OR OBJECTIVE | PAGES |
|------------------------------|----------------------|------------------------------|-------|
|------------------------------|----------------------|------------------------------|-------|

IC50s: BMS-515832-02-002; 280429; 280587

CaR response in T1 cells

49513-079
49513-088

49513 075

NOTEBOOK No. PAGE

CaR response in T11 cells

T11 cells plated out at 24,000 cells/well used (see also 49513-068).
0.8 mM Ca^{++} basal, 1.7 mM Ca^{++} stimulation.
See also 44676-072 for basic protocol.

Plate 1

BMS-515832:02-002 (uM) (A1-C6)

synthesis

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|--------|--------|--------|--------|--------|--------|
| A | 1.0000 | 0.3333 | 0.1111 | 0.0370 | 0.0123 | 0.0041 |
| B | 1.0000 | 0.3333 | 0.1111 | 0.0370 | 0.0123 | 0.0041 |
| C | 1.0000 | 0.3333 | 0.1111 | 0.0370 | 0.0123 | 0.0041 |

ATTACHMENT M

SIGNED

DATE

WITNESSED AND UNDERSTOOD BY:

DATE

Zheng Ma

[Signature]

NOTEBOOK No.

PAGE

Signal Test

Continued

Site

Plate 1 ZMCA072601a_n0

Minimum 9045.6 -16.47%

Maximum 12823.2

Average 10829.4

STDEV: 738.3

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---------|---------|---------|---------|---------|---------|
| A | 11194.4 | 10826.4 | 10314.4 | 10444.0 | 10192.8 | 9940.0 |
| B | 10764.0 | 11074.4 | 10508.0 | 9893.6 | 9832.8 | 9298.4 |
| C | 9922.4 | 11116.0 | 10592.8 | 10845.6 | 10831.2 | 10120.8 |

ATTACHMENT
IV

SIGNED

Zhengyi Ma

DATE

WITNESSED AND UNDERSIGNED BY

DATE

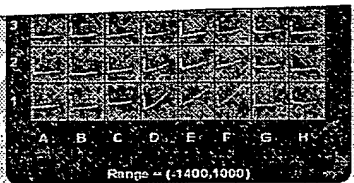
9513 03

NOTEBOOK No.

PAGE

Continued

ZMCo072501o_nt.fid



ATTACHMENT O

Range = (-1000,19000)

WITNESSED AND UNDERSTOOD BY:

DATE

Zhengji Ma

[Signature]

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

49513 083

NOTEBOOK No. PAGE

Continued

File = D:\nash\ZMCs072601a_n1.fid

Plate 1

Statistic = Max - Min

Start Sample = 11

End Sample = 45

Positive Scaling = On

Negative Correction = Off

Bias Value Subtract = On Spatial Uniformity Correction = On

Bias Sample = 1

| | A | B | C | D | E | F | G | H |
|---|-------|-------|------|-------|-------|-------|-------|-------|
| 1 | 3.79 | 4.99 | 2.39 | 43.82 | 15.29 | 26.01 | 2.4 | 3.96 |
| 2 | 4.56 | 4.47 | 3.85 | 60.51 | 45.13 | 65.27 | 4.92 | 3.28 |
| 3 | 11.06 | 12.77 | 17.3 | 95.26 | 62.57 | 92.4 | 20.93 | 11.91 |

ATTACHMENT P

SIGNED

Zhenyig Ma

DATE

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49513 084

NOTEBOOK No. PAGE

Continued

Test Occasion ID: MDCaR010726-1
Protocol ID: CaR-H-ICS0
Study ID: CaR
User ID: Zhengping Ma

Plate 1

| Compound ID | Conc (µM) | % TL1 | % TL2 | % TL3 | Avg % TL | StDev | IC50 |
|-------------------|-----------|-------|-------|-------|----------|-------|----------|
| BMS-515632-02-002 | 1.000 | 3.79 | 4.98 | 2.39 | 3.72 | 1.30 | 0.024921 |
| | 0.333 | 4.56 | 4.47 | 3.85 | 4.29 | 0.39 | SHRIL |
| | 0.111 | 11.06 | 12.77 | 17.3 | 13.71 | 3.22 | -1.15 |
| | 0.037 | 32.3 | 43.95 | 44.12 | 40.14 | 6.79 | |
| | 0.012 | 58.61 | 48.98 | 99.99 | 68.53 | 27.51 | |
| | 0.004 | 41.18 | 61.95 | 37.58 | 46.90 | 13.16 | |



SIGNED

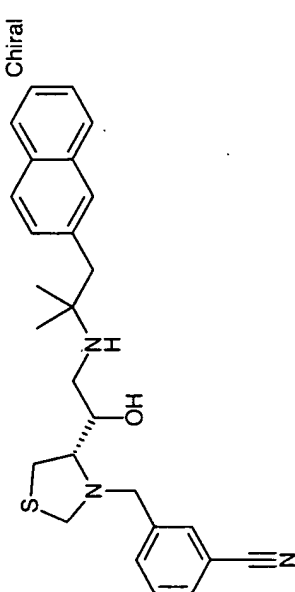
Zhengping Ma

DATE

WITNESSED AND UNDERSTOOD BY:

DATE

ATTACHMENT Q

| 2D | 3D | Biology | CTR | HTS | Inventory | Library | Program | Property | Reaction | Reagent | Select | Info | |
|---|----|---------|-----|-----|-----------|---------|---|----------|--------------------|---------|----------------------|------|--|
|  <p>Chiral</p> | | | | | | | | | | | | | |
| BMS # | | | | | | | BMS-515832 | | Form | | 02 | 002 | |
| Formula | | | | | | | C ₂₇ H ₃₁ N ₃ O ₃ S | | Appearance | | off-white powder | | |
| Submitter | | | | | | | Chen, Ying | | Chemist's Notebook | | Amount (MG) | | |
| Date | | | | | | | | | 48255-108-20 | | Purity | | |
| | | | | | | | | | Molname | | isomer B | | |
| Protocol | | | | | | | CaR_H_IC50 | | Version | | 1 | MAZH | |
| Protocol Name | | | | | | | Ca+ Sensitive Receptor, Human species, Dose Response assay | | Biologist | | Biologist's Notebook | | |
| Parameter | | | | | | | RECEPTOR SPECIES | | Assoc Value | | Ca SENSITIVE HUMAN | | |
| | | | | | | | | | MD_PPAR | | PWG List Name | | |
| | | | | | | | | | Result Comments | | | | |

| Library Name | Test Occasion | Test Occasion Notes | Parent_Study | Condition 1 | Condition 2 | Condition 3 | Condition 4 |
|--------------|---------------|---------------------|--------------|-------------|-------------|-------------|-------------|
| | MDCaR010726-1 | | | | | | Name |
| | MDCaR010726-1 | | | | | | NValue |
| | MDCaR010726-1 | | | | | | Units |
| Alliance ID | MDCaR010809 | | | | | | |
| | MDCaR010816 | | | | | | |
| | MDCaR010816 | | | | | | |

| Study | Protocol | V | Type | AValue | </> | NValue | Units | C | C 1 AValue | C 2 AValue | C 3 AValue | C 4 AValue | Date |
|-------|------------|---|-----------|----------|-----|---------|-------|---|------------|------------|------------|------------|------|
| CaR | CaR_H_IC50 | 1 | HILL | -1.15 | | -1.1500 | | | | | | | |
| CaR | CaR_H_IC50 | 1 | IC50 | 0.024921 | | 0.0249 | uM | | 0.33 | | | | |
| CaR | CaR_H_IC50 | 1 | P_CONTROL | 4.29 | | 4.2900 | | | | | | | |

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